Intestinal Mucosal Mast Cell Immune Response and Pathogenesis of Two *Eimeria acervulina* Isolates in Broiler Chickens

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ABSTRACT Four experiments were conducted comparing intestinal immune responses to 2 isolates of *Eimeria acervulina* (EA), EA1 and EA2. In experiments 1 and 2, broiler chicks of 2 commercial breeds were divided into control (nonchallenged), EA1-, or EA2-challenged groups. On d 6 postchallenge (PC), changes in BW were determined, intestinal lesions were scored, and duodenal tissue was evaluated for morphometric alterations and mucosal mast cell numbers. EA1 produced classical duodenal lesions and reduced villus height to crypt depth ratios compared with controls; however, no differences were found in mast cell counts. EA2 produced different results, and observed data were suggestive of an anaphylactic-like intestinal secretory response compared with EA1 or controls. In experiment 3, tissues were analyzed from d 2 through 6 PC. Villus atrophy and crypt hyperplasia were increased on d 5 PC in both challenged groups. Mast cell counts were significantly greater on d 3 and 4 PC in EA1-challenged birds. In experiment 4, EA2 oocysts were cleaned with 5.25% sodium hypochlorite to evaluate the possibility of a bacterial contaminant contributing to the pathogenesis of intestinal alterations. No evidence of a bacterial contaminant contributing to the pathology was observed. These data are indicative of differential host response and immunovariability between different isolates of the same *Eimeria* species in 2 breeds of commercial broiler chickens.

(*Key words*: broiler, *Eimeria*, immunity, immunovariability, mast cell)

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INTRODUCTION

Infections caused by coccidial parasites have had a major economic impact on the US commercial poultry industry in the past several decades, with reports of 1 to 2 billion dollars in annual losses relating to coccidial treatment, infection, and prevention (Danforth and Augustine, 1989; Talebi and Mulcahy, 1995; Yun et al., 2000). Coccidial infections induce a variety of pathological and immunological responses that help the host acquire protective immunity and defend against the parasite. It has been reported that immunological and nonimmunological defenses play a role at the intestinal mucosal surface during *Eimeria* invasion (Lillehoj and Trout, 1993). It has been difficult to completely understand these protective mechanisms due to continued emergence of drug-resistant strains and the complexity of the *Eimeria* species life cycle. Past studies have measured the severity of coccidiosis on the basis of weight gains, lesion scores, cessation of total oocyst output, and humoral and cellular responses (Stiff and Bafundo, 1993; Talebi and Mulcahy, 1995). A closer look at the histological and physiological changes resulting from infection at the mucosal level is pertinent to understanding the specific nature of this immunity.

Until recently, coccidia were classified according to morphological, physiological, and behavioral characteristics, such as those described by Brackett and Bliznick (1950). It is known that different isolates of *Eimeria* exist; however, little information is available comparing the immunogenicity and immunovariability of different strains within each *Eimeria* species. Shirley (1985) described new techniques for revealing definitive markers of different strains within the same species of *Eimeria*; however, the differential host response to the different strains was not discussed in detail. Different strains of *Eimeria* are capable of showing antigenic diversity, so that the immunity conferred by one strain does not completely protect chickens against further challenge by a different strain of the same species (Talebi and Mulcahy, 1995). In the reviews on the use of coccidial vaccines by Danforth and Augustine (1989) and Danforth (1998), such antigenic

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Abbreviation key: EA = *Eimeria acervulina*; EA1 = *E. acervulina* isolate 1; EA2 = *E. acervulina* isolate 2; PC = postchallenge.

diversity was supported by observations demonstrating isolates from different geographical areas that do not always show cross-protection following oral immunization. This diversity may be a challenging factor in producing specific vaccines for coccidial control programs. Opportunities for the development of alternative control measures may be elucidated through evaluation of variability in the host's protective immune response to different strains.

It is well established that B and T lymphocytes are involved in responses to *Eimeria* invasion, but there is limited data concerning the possible participation of other effector cells, such as mast cells, which may participate in protective immunity. It is reported that mast cell responses contribute to adaptive immunity in mammalian parasitic infections (Rose, 1982; Abraham and Arock, 1998), but their involvement in chickens has been largely overlooked. However, an acute mucosal mastocytophilia response has been reported during secondary *Eimeria* challenge in chickens (Rose et al., 1980). Whether this response involved an increase in mast cell numbers or whether cell migration occurred is in question (Daszak et al., 1993). Mast cells possess distinct attributes that support their role in immune responses, but researchers have been limited in their ability to accurately describe the presence of mast cells during inflammatory responses due to changes in staining properties and morphology associated with tissue location and species variation (Yong, 1997). If mast cells are responsible for aiding in protective immunity, more consistent and accurate identification is needed.

The interaction between *Eimeria* and the intestinal mucosal immune system is a key component in the defense of the chicken to these enteric pathogens. Before control strategies can be improved, it is important to further the present understanding of the host-parasite interaction and the possible role of effector cells in the immune response. The objectives of the experiments conducted were to compare the response of 2 breeds of commercial broilers to 2 isolates of *Eimeria acervulina* (EA) to determine if differences in BW gain or lesion scores exist between different strains within the same species of *Eimeria*, and to analyze the morphological intestinal host response to each, as measured by villus height, crypt depth, and mast cell numbers.

MATERIALS AND METHODS

Experimental Birds

In all experiments, straight-run, day-of-hatch broiler chicks of breed A or breed B were obtained from a local commercial hatchery. Chicks were placed on clean pine shavings (experiments 1, 2, and 4) or wire-floored starter batteries (experiment 3) and given ad libitum access to water and unmedicated starter feed, which met or exceeded NRC (1994) requirements. For the first 2 wk of life, all chicks were housed in the same room in a controlled environment of age-appropriate temperatures. At 14 d of age (d 15 for experiment 2), chicks were weighed, wing-banded for breed and bird identification, and assigned to nonchallenged or challenged treatment groups. Control birds were housed in a separate room (within the same building) from the challenge birds for isolation purposes. Rooms were monitored and maintained at the same ambient temperature. In all experiments, rooms were provided adequate negative pressure ventilation and birds were kept on continuous incandescent lighting.

Eimeria

Two EA isolates, *Eimeria acervulina* isolate 1 (EA1) and *Eimeria acervulina* isolate 2 (EA2) were supplied by Harry Danforth (USDA/ARS, Beltsville, MD) for use in the experiments. Oocysts were stored at 4°C in 2% potassium dichromate prior to challenge. Oocysts for challenge were centrifuged for 10 min at 4°C and $845 \times g$. The supernatant was removed, and the pellet was resuspended in PBS and centrifuged again using the same method. This process was repeated at least 3 times to remove the potassium dichromate. After the final centrifugation, the pellet was resuspended in sterile PBS for quantification of oocysts with a hemocytometer. Additional PBS was added to bring the inoculum to the desired challenge dose. Challenge concentrations for each experiment were determined by a dose titration experiment prior to the first trial and by the previous experimental results for subsequent trials. Challenged chicks received 1 mL of quantified sporulated oocysts by per os crop gavage on day of challenge.

Morphometric Analysis

Descending duodenal loop samples (~25 mm) were collected 6 d postchallenge (PC) for analysis of mast cell number and morphological changes, including villus height and crypt depth. Birds were killed by cervical dislocation, and the duodenal tissue from each broiler was immediately excised, rinsed with ice cold PBS, immersed in 10% neutral buffered formalin for 48 h for fixation, and then placed in 70% ethanol. Each tissue was cut into 5 sections for placement on each slide. Tissues were processed, embedded, sectioned at 4 μm, and stained with toluidine blue for the identification of mast cells and morphological measurements. Mast cell counts were made in the lamina propria region of the villus using an intraocular 5×5 eyepiece grid (total area = 156.25 μm^2) with the 40× objective of a bright-field microscope. Three complete tissues/slide and 15 grids/tissue were counted to quantify mast cells. Morphological changes were measured in millimeters using a bright-field microscope and the Sigma Scan Pro software program.[2] Three tissues/slide and 4 measurements/tissue were taken for changes in villus heights and crypt depths. Villi:crypt ratios were determined by average villus height measurements divided by crypt depth measurements.

[2]SPSS, Inc., Chicago, IL.

Experimental Design

Four experiments were conducted to evaluate the intestinal response to the 2 isolates of EA. In 3 of the 4 experiments, birds were challenged with 1 of 2 EA isolates, and data were collected to compare differences in BW gain, lesion scores, morphometric alterations, and mast cell influx into the upper small intestine. The fourth experiment was conducted to determine if the differential host response to one of the isolates was due to the parasite-host interaction itself, or a bacterial or fungal contaminant present in the challenge inoculum. In 2 of the experiments conducted, the responses of 2 commercial broiler breeds were compared to determine if there was a different degree of susceptibility or pathological response to the 2 isolates of EA.

Birds were identified with metal wing-bands and weighed individually on day of challenge and 6 d PC for determination of BW gain during the challenge. On d 6 PC, chicks were killed by cervical dislocation, and the duodenum of the upper small intestine was quickly removed and evaluated for EA lesion scores using the method described by Johnson and Reid (1970).

Experiment 1. Straight-run broiler chicks of 2 commercial breeds (breed A and breed B; n = 75/breed) were raised in 2 floor pens dependent on breed for the first 14 d posthatch. On d 14 (d 0 PC), birds were separated into 3 treatment groups with an equal number of breed A and B (n = 25 birds/breed per treatment) within each group. Challenged groups received 1.50×10^5 oocysts/mL of EA1 or 1.75×10^5 oocysts/mL of EA2 per os, respectively. On d 6 PC, all birds were weighed, lesions were scored, and duodenal samples were taken for histological examination.

Experiment 2. Experiment 2 differed from experiment 1 in that birds were challenged with 2.0×10^5 oocysts/mL of EA1 or 2.5×10^5 oocysts/mL of EA2 on d 15 rather than d 14. On d 6 PC, all birds were weighed, lesions were scored, and duodenal samples were taken for histological examination.

Experiment 3. Experiment 3 was conducted to evaluate the time-course (d 2 to 6 PC) of the intestinal mast cell response and pathogenesis in response to EA1 and EA2 challenges. Straight-run broiler chicks of breed A were raised in starter batteries[3] (n = 20/cage) with feed and water ad libitum. On d 14, chicks were randomly distributed into 3 experimental groups (n = 66/treatment) and placed in grower batteries (n = 22/cage; 3 replicate pens/treatment) with controls and challenged groups housed in separate rooms. Challenged groups received 3.0×10^5 oocysts/mL of EA1 or EA2 per os. New harvests of EA1 and EA2 were used in this experiment to ensure an infective state. Daily from d 2 to 6 PC, 9 birds per treatment were euthanized and samples of duodenal tissue were taken. On d 6 PC, all remaining birds (n = 30/treatment) were scored for duodenal lesions.

[3]Petersime NV, Centrumstraat 125, B-9870 Zulte, Belgium.

Experiment 4. Experiment 4 was conducted to determine if bacterial or fungal contamination of the EA2 inoculum was responsible for the observed differential host response. Oocysts for challenge were prepared using the following cleaning procedure. Four mL of EA2 oocysts, as used for previous challenge were placed in 15mL conical tubes and centrifuged twice at $845 \times g$ for 10 min with supernatant removed and oocysts resuspended between centrifugations. The oocyst pellet was cleaned with 5.25% sodium hypochlorite or double distilled H_2O at the ratio of 1.5 times the pellet volume and agitated. The oocysts were transferred to 50-mL conical tubes and maintained at 4°C for 30 min, with gentle swirling at 10-min intervals. The tubes were then filled with double distilled H_2O and centrifuged for 15 min at 4°C and $1{,}320 \times g$. The supernatant was removed and centrifugation was repeated 5 times. The pellet was resuspended with sterile PBS, and oocysts were quantified using a hemocytometer. Sterile PBS was added to bring the inoculum to the desired challenge dose of 2.5×10^5 oocysts/mL.

Straight-run broiler chicks (n = 60) of breed A were placed in floor pens for the first 14 d posthatch. On d 14, birds were randomly separated into 3 treatment groups consisting of control (nonchallenged), EA2 group 1 (challenged with 2.5×10^5/mL of 5.25% sodium hypochlorite-washed oocysts) and EA2 group 2 (challenged with 2.5×10^5/mL of double distilled H_2O-washed oocysts). On d 6 PC, duodenal samples were collected, and lesions were scored for all birds.

Statistical Analysis

Weight gains, duodenal lesion scores, and morphometric alteration data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1998). Mast cell counts were log transformed prior to analysis and then converted to original scale using the inverse log function. The least square means procedure of SAS was used to obtain the equivalent of Tukey's honestly significant difference comparisons (SAS Institute, 1998). All results are reported at the $P \leq 0.05$ level, unless otherwise indicated.

RESULTS

Experiment 1

Experiment 1 compared the effects of EA1 and EA2 on 2 commercial broiler breeds. Results of experiment 1 (Table 1) indicated no significant differences between EA2-challenged birds and nonchallenged controls. Though not always significant, weight gains were lower in breed B compared with breed A. Although there were no lesions present in EA2-challenged birds, the birds were observed to have watery intestinal contents. Lesions of the intestinal mucosa were observed within both breeds challenged with EA1, of which breed A had lower average lesion scores than breed B. Differences in villus height were observed, with villi significantly shorter in EA1-challenged birds compared with EA2-challenged or non-

TABLE 1. Weight gain (WG), duodenal lesion score, morphometric alterations, and mast cell response 6 d postchallenge with *Eimeria acervulina* (EA) (experiment 1)

Treatment/breed[1]	WG (g)[2]	Lesion score[3]	Villus height (mm)[2]	Crypt depth (mm)[2]	Villus:crypt ratio[2]	Mast cells (156.25 μm^2)		
						LCB[4]	Mean	UCB[5]
Control/A	241.38 ± 6.42^{ab}	0^{c}	1.87 ± 0.06^{a}	0.23 ± 0.01^{b}	8.47 ± 0.39^{a}	0.03	0.10^{a}	0.33
EA1/A	262.48 ± 6.55^{a}	2.08 ± 0.11^{b}	1.57 ± 0.06^{b}	0.31 ± 0.01^{a}	5.15 ± 0.39^{b}	0.08	0.27^{a}	0.90
EA2/A	262.92 ± 6.68^{a}	0^{c}	1.83 ± 0.06^{a}	0.20 ± 0.01^{b}	9.20 ± 0.39^{a}	0.07	0.23^{a}	0.79
Control/B	221.84 ± 6.55^{b}	0^{c}	1.71 ± 0.06^{ab}	0.22 ± 0.01^{b}	7.85 ± 0.39^{a}	0.19	0.63^{a}	2.12
EA1/B	223.43 ± 7.14^{b}	2.43 ± 0.12^{a}	1.51 ± 0.06^{b}	0.28 ± 0.01^{a}	5.41 ± 0.39^{b}	0.11	0.36^{a}	1.22
EA2/B	237.81 ± 6.98^{ab}	0^{c}	1.66 ± 0.06^{ab}	0.20 ± 0.01^{b}	8.47 ± 0.39^{a}	0.17	0.56^{a}	1.88
Treatment × breed	$P \leq 0.001$	$P \leq 0.01$	$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.001$	—	NS	—

[a–c]Means in columns with no common superscript differ significantly ($P \leq 0.05$).

[1]Treatments: Control = no challenge; EA1 = 1.50×10^5 EA isolate 1 oocysts; EA2 = 1.75×10^5 EA isolate 2 oocysts; Breed = A or B.

[2]Values are means ± SEM.

[3]Duodenal lesion score from 0 to 4.

[4]LCB = Lower confidence bound limit of the 95% confidence interval.

[5]UCB = Upper confidence bound limit of the 95% confidence interval.

challenged groups, particularly in Breed A. Birds challenged with EA1 also had deeper crypts, which in association with shorter villi, resulted in a significantly decreased villus:crypt ratio compared with control or EA2-challenged birds. There were no differences in mast cell counts.

Experiment 2

Experiment 2 compared the pathophysiology of EA1 and EA2 on 2 commercial broiler breeds (Table 2). Infection with EA2 produced significant differences compared with EA1 or controls, including lower weight gains, lesion scores, villus height, and villus:crypt ratios, as well as an increase in crypt depth. No significant differences in mast cell counts were observed in this experiment. EA1-challenged birds appeared healthy and exhibited no clinical signs, whereas EA2-challenged birds appeared listless and in a moribund condition. White plaque-like intestinal lesions were prominent in EA1-challenged birds; however EA2-challenged birds exhibited few classical lesions. In the EA2-challenged birds there was evidence of sloughing of the intestinal mucosa and the intestines were pale and friable.

Experiment 3

To follow the time course of the mucosal responses to EA1 or EA2, experiment 3 compared morphological alterations and mast cell influx on d 2 through 6 PC, and weight gain and lesion scores on d 6 PC in breed A broiler chickens. Weight gains of birds challenged with EA1 were decreased compared with controls (Table 3), whereas weight gains of birds challenged with EA2 were not different than controls. No differences were found in lesion scores between EA1- and EA2-challenged birds, which were both significantly increased compared with controls (no lesions). Measurements taken from duodenal tissue of EA1 birds 2 to 6 d PC revealed a rapid decrease in villus height, reaching a minimum height on d 5, at which time significant differences were found when compared with control or EA2-challenged groups. On d 6 PC, villus height in EA1-challenged birds was not significantly different than that in EA2-challenged birds but was lower

TABLE 2. Weight gain (WG), duodenal lesion score, morphometric alterations, and mast cell response 6 d postchallenge with *Eimeria acervulina* (EA) (experiment 2)

Treatment/breed[1]	WG (g)[2]	Lesion score[3]	Villus height (mm)[2]	Crypt depth (mm)[2]	Villus:crypt ratio[2]	Mast cells (156.25 μm^2)		
						LCB[4]	Mean	UCB[5]
Control/A	334.08 ± 9.54^{a}	0^{c}	2.01 ± 0.06^{a}	0.22 ± 0.02^{b}	9.43 ± 0.46^{a}	0.20	0.54^{a}	1.48
EA1/A	339.90 ± 9.76^{a}	1.29 ± 0.13^{ab}	1.83 ± 0.06^{ab}	0.22 ± 0.02^{b}	8.27 ± 0.46^{a}	0.15	0.40^{a}	1.09
EA2/A	202.28 ± 10.20^{b}	1.03 ± 0.13^{b}	1.22 ± 0.06^{c}	0.36 ± 0.02^{a}	3.48 ± 0.46^{c}	0.50	1.38^{a}	3.78
Control/B	306.80 ± 9.60^{a}	0^{c}	1.78 ± 0.06^{ab}	0.23 ± 0.02^{b}	8.62 ± 0.46^{a}	0.37	1.02^{a}	2.80
EA1/B	326.88 ± 10.18^{a}	1.60 ± 0.13^{a}	1.73 ± 0.06^{b}	0.23 ± 0.02^{b}	7.53 ± 0.46^{ab}	0.16	0.44^{a}	1.21
EA2/B	192.11 ± 10.33^{b}	0.93 ± 0.13^{b}	1.01 ± 0.06^{c}	0.37 ± 0.02^{a}	2.72 ± 0.46^{c}	0.24	0.67^{a}	1.84
Treatment × breed	$P \leq 0.0001$	$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.0001$	$P \leq 0.0001$	—	NS	—

[a–c]Means in columns with no common superscript differ significantly ($P \leq 0.05$).

[1]Treatments: Control = no challenge; EA1 = 2.0×10^5 EA isolate 1 oocysts; EA2 = 2.5×10^5 EA isolate 2 oocysts; Breeds = A or B.

[2]Values are means ± SEM.

[3]Duodenal lesion score from 0 to 4.

[4]LCB = Lower confidence bound limit of the 95% confidence interval.

[5]UCB = Upper confidence bound limit of the 95% confidence interval.

TABLE 3. Weight gain (WG), duodenal lesion score, morphometric alterations, and mast cell response 2 to 6 d postchallenge with *Eimeria acervulina* (EA) (experiment 3)

d PC[1]	Treatment[2]	WG (g)[3]	Lesion score[4]	Villus height (mm)[3]	Crypt depth (mm)[3]	Villus:crypt ratio[3]	Mast cells (156.25 μm^2)		
							LCB[5]	Mean	UCB[6]
2	Control	NA[7]	NA	1.63 ± 0.08[a]	0.19 ± 0.01[a]	8.48 ± 0.46[a]	1.48	2.46[a]	4.10
	EA1	NA	NA	1.80 ± 0.08[a]	0.23 ± 0.01[a]	8.04 ± 0.46[a]	1.72	2.86[a]	4.76
	EA2	NA	NA	1.65 ± 0.08[a]	0.20 ± 0.02[a]	8.38 ± 0.46[a]	1.95	3.25[a]	5.42
3	Control	NA	NA	1.71 ± 0.08[a]	0.21 ± 0.01[a]	8.62 ± 0.46[a]	1.13	1.88[b]	3.13
	EA1	NA	NA	1.85 ± 0.08[a]	0.24 ± 0.01[a]	8.11 ± 0.46[a]	2.64	4.39[a]	7.32
	EA2	NA	NA	1.78 ± 0.08[a]	0.22 ± 0.01[a]	8.05 ± 0.46[a]	1.25	2.08[ab]	3.46
4	Control	NA	NA	1.74 ± 0.08[a]	0.19 ± 0.01[b]	9.36 ± 0.46[a]	1.36	2.27[b]	3.78
	EA1	NA	NA	1.62 ± 0.08[a]	0.25 ± 0.01[a]	6.55 ± 0.46[b]	3.49	5.81[a]	9.68
	EA2	NA	NA	1.77 ± 0.08[a]	0.24 ± 0.01[a]	7.67 ± 0.46[ab]	2.32	3.86[ab]	6.42
5	Control	NA	NA	1.77 ± 0.08[a]	0.20 ± 0.01[c]	9.15 ± 0.46[a]	0.94	1.57[a]	2.61
	EA1	NA	NA	1.37 ± 0.08[b]	0.36 ± 0.01[a]	3.92 ± 0.46[c]	2.69	4.48[a]	7.46
	EA2	NA	NA	1.67 ± 0.08[a]	0.29 ± 0.01[b]	5.78 ± 0.46[b]	1.75	2.92[a]	4.85
6	Control	327.36 ± 7.48[a]	0[b]	1.91 ± 0.08[a]	0.20 ± 0.01[c]	9.50 ± 0.46[a]	1.67	2.77[a]	4.62
	EA1	300.52 ± 7.62[b]	1.74 ± 0.10[a]	1.58 ± 0.08[b]	0.38 ± 0.01[a]	4.30 ± 0.46[b]	3.60	5.99[a]	9.97
	EA2	313.43 ± 7.48[ab]	1.43 ± 0.09[a]	1.68 ± 0.08[ab]	0.32 ± 0.01[b]	5.28 ± 0.46[b]	3.46	5.75[a]	9.58
d PC × treatment		$P \leq 0.05$	$P \leq 0.0001$	$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.01$	—	$P \leq 0.05$	—

[a–c]Means in columns with no common superscript differ significantly within day ($P \leq 0.05$).

[1]d PC = day postchallenge.

[2]Treatments: Control = no challenge; EA1 = 3.0×10^5 EA isolate 1 oocysts; EA2 = 3.0×10^5 EA isolate 2 oocysts.

[3]Values are means ± SEM.

[4]Duodenal lesion score from 0 to 4.

[5]LCB = Lower confidence bound limit of the 95% confidence interval.

[6]UCB = Upper confidence bound limit of the 95% confidence interval.

[7]NA = not assessed.

than controls. Duodenal crypt depths were increased by EA infection on d 4 through 6 PC compared with controls, with EA1-challenged birds having significantly deeper crypts than EA2-challenged birds on d 5 and 6 PC. Villus height:crypt depth ratio was lower in challenged birds than controls on d 4 through 6 PC, with the exception of EA2-challenged birds on d 4 PC, which was not significantly different. There were no differences between EA1- and EA2-challenged birds prior to d 5 PC, at which point EA1-challenged birds had significantly lower villus heights, increased crypt depths, and hence, an overall lower villus height:crypt depth ratio. Mast cell counts for EA1-challenged birds were significantly greater than controls on d 3 and 4 PC; however, EA2 mast cell counts were not significantly different from EA1 or controls on any day sampled. Observational data revealed responses to EA1 and EA2 were quite different in this experiment than the other trials conducted. In experiment 3, there appeared to be a secretory response in EA1-challenged birds, and a similar, but less severe response in EA2 -challenged birds.

Experiment 4

To evaluate the possibility of a bacterial or fungal contaminant in the EA2 challenge inocula that might contribute to the differential host response and pathology, experiment 4 compared the effects of inoculating birds with EA2 oocysts washed with double distilled H_2O or 5.25% sodium hypochlorite. Weight gains of challenged birds were found to be lower than controls for the disinfected and normal oocyst preparations (Table 4). Lesions were present only in challenged birds, with no significant differences between cleaned EA2 and EA2 as used for other experiments. Intestinal contents of challenged birds were watery with sloughed epithelium present in the lumen. Although not analyzed, the duodenum in challenged birds appeared pale with a weakened intestinal strength compared with controls. Villus height decreased, crypt depth increased, and villus height:crypt depth ratios decreased in both challenged groups compared with controls, with no differences present between challenged groups. No differences in mast cell counts were observed in this experiment.

DISCUSSION

The present experiments were conducted to evaluate variability in the broiler host response to a single challenge with 1 of 2 EA isolates, and to determine if responses were influenced by the genetics of the bird. These experiments demonstrated that immunovariability does exist between different isolates within the same species of *Eimeria*. Differences in PC BW gains, duodenal lesion scores, villus height, and crypt depth were dependent upon the strain administered; however, quantified and observed effects were not always consistent and may have been influenced by the challenge dosages of EA.

Evidence has indicated that genetics have a major role in the bird's immune function and ability to cope with coccidial parasites (Lillehoj, 1988; Uni et al., 1995). With the exception of experiment 3, weight gains in EA1-chal-

TABLE 4. Weight gain (WG), duodenal lesion score, morphometric alterations, and mast cell response 6 d postchallenge with *Eimeria acervulina* (EA) (experiment 4)

Treatment[1]	WG (g)[2]	Lesion score[3]	Villus height (mm)[2]	Crypt depth (mm)[2]	Villus:crypt ratio[2]	Mast cells (156.25μm^2)		
						LCB[4]	Mean	UCB[5]
Control	287.17 ± 10.38[a]	0[b]	1.79 ± 0.06[a]	0.19 ± 0.01[b]	9.72 ± 0.34[a]	0.76	1.58[a]	3.32
EA2C	250.90 ± 9.65[b]	1.31 ± 0.09[a]	1.14 ± 0.06[b]	0.32 ± 0.01[a]	3.61 ± 0.34[b]	0.32	0.67[a]	1.4
EA2U	221.39 ± 10.04[b]	1.28 ± 0.10[a]	1.07 ± 0.06[b]	0.33 ± 0.01[a]	3.28 ± 0.34[b]	0.66	1.38a	2.89
Treatment	$P \leq 0.05$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	—	NS	—

[a–b]Means in columns with no common superscript differ significantly ($P \leq 0.05$).

[1]Treatments: Control = no challenge; EA2C = EA isolate 2 oocysts cleaned with 5.25% sodium hypochlorite (2.0×10^5 oocysts); EA2U = EA isolate 2 oocysts cleaned with double distilled H_2O (2.5×10^5 oocysts).

[2]Values are means ± SEM.

[3]Duodenal lesion score from 0 to 4.

[4]LCB = Lower confidence bound limit of the 95% confidence interval.

[5]UCB = Upper confidence bound limit of the 95% confidence interval.

lenged birds, were not significantly different from weight gain of controls. In contrast, EA2 challenge significantly depressed weight gains when compared with controls in experiments 2 and 4, but not in experiments 1 or 3. This discrepancy in weight gain response between experiments could be attributed to insufficient oocyst challenge or aging of the inocula (Ruff et al., 1981). Studies conducted using homologous EA challenges have also indicated differing weight gain results. Ogbuokiri and Edgar (1985) and McKenzie et al. (1987) reported that no weight gain differences were detected between challenged birds and controls; whereas Stephens et al. (1974) reported depressed weight gains with EA inoculation. These differences in weight gains are not surprising because many factors are involved in *Eimeria* infection, such as a disruption of the intestinal mucosa, compromised nutrient absorption, and accelerated feed passage through the digestive system (Jeurissen et al., 1996; McDougald and Reid, 1997; Yun et al., 2000). In addition, the degree of infection, the amount of water and feed consumption (Williams, 1996), the strain of EA, and the inoculating dosage are all factors contributing to differential weight gains (Long and Johnson, 1988; Graat et al., 1996). However, the immune system has a high demand for nutrients while combating an infection, and therefore, generation of an immune response could be a direct contributor to impaired growth. Variations in experimental design and the factors stated above could, singly or in combination, have an influence on host responses to weight gains in an *Eimeria* infection.

In all experiments conducted, lesions of the duodenal mucosa of EA1-challenged birds consisted of typical white, ladder-like plaques, which were consistent with the findings of Johnson and Reid (1970), upon which the current method of lesion scoring was based. However, EA2 challenge produced significantly fewer classical lesions but exhibited signs of an anaphylactic-like secretory-type response. In experiments 1 and 3, these findings were not consistent. In experiment 1, there were no intestinal lesions in EA2-challenged birds, possibly due to the age of the challenge isolate. In experiment 3, EA1-challenged birds exhibited some clinical signs and intestinal characteristics observed in EA2-challenged birds in the other experiments. Interestingly, the alterations in lesion appearance in experiments 1 and 3 correspond to the contrasting results of BW gain in these experiments. Breed differences in lesion scores were only found in experiment 1, with breed A having significantly lower lesion scores, which supports the fact that genetics play a role in immune status (Lillehoj, 1988; Lillehoj and Trout, 1993; Yun et al., 2000). Differences in lesion scores found between EA1- and EA2-challenged birds within the same breed could be related to the fact that the 2 isolates are generating a differential host response due to the presence of different surface antigens, which may or may not cause edema and the presence of intestinal lesions (Lillehoj and Trout, 1993). Similar findings suggest that immunological mechanisms responsible for protecting birds against weight loss may differ from mechanisms that protect against lesions of the intestinal mucosa (Augustine et al., 1991).

Coccidiosis is known to produce different histopathological features depending on the *Eimeria* species causing the infection (Johnson and Reid, 1970; Lillehoj and Trout 1993; McDougald and Reid, 1997). However, examination of the duodenal tissues taken from EA-challenged birds 6 d PC confirmed a possible differential host response to different isolates as well. Although experiment 4 eliminated the likelihood that a bacterial or fungal contaminant was responsible for the differential response, the possibility remains that the host response was altered by a viral contaminant, such as chicken anemia virus, which would not be removed by the sodium hypochlorite treatment. Isolate EA1 significantly reduced villus lengths compared with controls, except in experiment 2. Birds challenged with EA2 had altered villus heights, although these findings were not consistent in all experiments. EA1 produced results comparable to those in experiments conducted by Fernando and McCraw (1973) for a single infection with EA. In the time-course sampling experiment, no differences in villus height were found between control and EA1-challenged birds until d 5 and 6 PC, which coincides with the height of infection (Fernando and McCraw, 1973). In EA2-challenged birds, histological examination revealed sloughing of the intesti-

nal lining exposing the lamina propria. The intestinal epithelium acts as a selective barrier, allowing nutritional ions and macromolecules to be absorbed, and resisting harmful pathogens. Once this protective barrier is removed, the exposed lamina propria undergoes morphological changes due to the effects of inflammation. The villus is then unable to adequately absorb nutrients and is subject to secondary infections (Schat and Myers, 1991). At the mucosal level, the villus structure may be altered directly by the infectious agent, or indirectly by the host's inflammatory response (Barker, 1993). Crypt hyperplasia is a well-known histopathologic consequence of coccidiosis, and results in increased metabolic activity to compensate for epithelial destruction (Rose et al., 1992). In the present experiments, observed increases of intestinal crypt depth were in agreement with results from experiments conducted by Fernando and McCraw (1973). Crypt hyperplasia was found to be present in EA1-challenged birds in all experiments except experiment 2, when compared with controls. Crypt length increased to a maximum at 6 d PC for both challenged groups. It appeared that the crypt measurements were inversely related to villus height in primary EA infection, and that severe alterations coincided with the heightened period of infection. Results from the present experiments indicated that the host responded to EA2 in a different manner than EA1 at the level of the duodenal mucosa.

New epithelial cells are produced in the intestinal mucosal crypts and migrate in an orderly fashion along the villi to the tips where cells desquamate (Schat and Myers, 1991). During infection, this metabolic process could be enhanced to protect the exposed lamina propria and to replace infected or damaged cells. Allen (1983) noted a significant increase in metabolic activity and an increase in lower intestinal lengths in chickens, which support the fact that compensatory gain of BW does occur during a heavy EA infection. Data from the present study suggested that during infection the increased crypt depths were directly related to increased replacement or turnover of epithelial cells, which suggest the host's intestinal response mechanism was trying to compensate for the effects of EA infection. These histopathological changes were evident by increases in villus height:crypt depth ratios, which correlate to the amount of villus atrophy and crypt hyperplasia during EA infection.

From these results, it is suggested that there was a differential immune response elicited by the 2 strains of EA. However, the mechanisms responsible for these acute differences are unknown. Arguably, the differential responses could have been due to the parasite itself, the host's reaction to the parasite, a combination of these, or alternatively, an unknown contaminant. It has been established that many cells are involved in responses to *Eimeria* at the site of invasion, including lymphocytes, large mononuclear cells, macrophages, granulocytes, and other leukocyte subpopulations (Rose et al., 1979; Rose, 1982; Jeurissen et al., 1996). However, mast cells have often been excluded from the analysis. Mast cells are considered the major effector cells of allergic reactions, but they have other immune functions, which are believed to be independent of IgE antibodies. Although the pivotal role of mast cells in nematode and allergic reactions is well established, the observations presented in this paper are inconclusive to suggest that mast cells may also have a role in the induction of specific immune responses to *Eimeria* challenge. Evaluation of mast cell responses 6 d PC in experiments 1, 2, and 4 revealed no significant differences in mast cell counts between treatments. However, in experiment 3, increases in mast cells were found on d 3 and 4 PC. These results suggest that, by sampling only on d 6 PC in the initial experiments, a change in mast cell number may have been missed. However, the increase in mast cell number observed in experiment 3 is only a preliminary indication of the involvement of mast cells and requires further investigation to be indicative of any role of mast cells in the immune response to EA. Inconsistencies in mast cell counts and the high variability seen could have several explanations. It is possible that certain mast cells in these experiments were indistinguishable from other cells due to degranulation. Mast cells vary from one host to another, and unless appropriate fixatives are used, the granules could be dissolved or otherwise lose their ability to take up stain (Tung, 1991). It is possible that mast cells may have released their contents before or during fixation of the tissues, and thus did not absorb the stain due to the absence of the granules. Mast cell appearance varied in size, shape, and number of granules present, which is suggestive of partial release of mast cell granules. Past studies suggest that mast cells release their granules shortly after antigenic stimulation, therefore making it difficult to count mast cells during an infective state (Huntley et al., 1985).

Although there is limited evidence of the role of mast cell products contributing to the pathophysiology of avian infectious diseases, their capacity to inflict damage is clearly significant in other species. Inflammatory responses mediated through the host may allow the parasite to use the host's resources to aid in its survival. Abraham and Arock (1998) report that successful pathogens evade and resist the host's inflammatory responses to foster their life cycle and proliferate. This concept could be applied to coccidiosis. It is conceivable that EA activates mast cells, hence contributing to anaphylaxis of the intestine involving excessive fluid and electrolyte secretion resulting in diarrhea. Consequently, the parasite is able to pass through the host, virtually unharmed, and disseminate itself throughout the environment, enabling uptake by other hosts. Improvements of effective coccidia vaccines and other means of controlling coccidiosis ultimately depend upon understanding the bird's immune system and its response to this parasitic infection. The present experiments reveal that immunovariability does exist within *Eimeria* species due to a differential host intestinal mucosal immune response, which further complicates understanding the induction of complete immunity to the pathogen and the differences in the pathogenesis of *Eimeria*.

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